

Evaluation of Acridine Orange Staining as a Replacement of Subcultures for BacT/ALERT-Positive, Gram Stain-Negative Blood Cultures

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Received 13 June 2003/Returned for modification 11 July 2003/Accepted 20 August 2003

Among 18,424 blood culture sets processed during a study period of 18 months, 85 bottles that were positive by the BacT/ALERT system were Gram stain negative. Both acridine orange staining and subcultures detected microorganisms in a total of 12 bottles. Acridine orange staining can replace subcultures of false-positive blood cultures.

Rapid detection of microorganisms in blood cultures is one of the most important tasks of the clinical microbiology laboratory. Modern automated blood culture systems have increased the isolation rate of microorganisms and shortened the time to detection. The BacT/ALERT system (BioMerieux Inc., Durham, N.C.) is based on the colorimetric detection of CO₂ produced by the growing microorganisms. The culture medium, detection system, and detection algorithm are designed to maximize the sensitivity of the system. A detection algorithm which maximizes sensitivity invariably leads to a certain percentage of false-positive results, i.e., cultures that are reported as positive by the system although no microorganisms can be detected by Gram staining or subcultures. Subculturing false-positive blood culture bottles is time-consuming and costly. Acridine orange (AO) staining has been described as a replacement for subculturing false-positive blood cultures with the BACTEC NR 660 system (1, 2). However, it is not possible to apply these findings to the BacT/ALERT system, since BacT/ALERT FA and FN bottles contain absorbent charcoal particles, which make the interpretation of Gram-stained and AO-stained smears difficult (7). In order to assess the need to perform routine subcultures of false-positive blood cultures with the BacT/ALERT system, we undertook a prospective study of all blood cultures that were reported as positive by the system but were Gram stain negative.

BacT/ALERT FA aerobic and BacT/ALERT FN anaerobic blood culture bottles were incubated in the BacT/ALERT Classic instrument for 7 days. From each bottle reported positive by the system an aliquot of broth was Gram stained. Before the Gram staining the slides were heat fixed. AO stain (Difco Becton Dickinson, Sparks, Mass.) was performed on all BacT/ALERT-positive, Gram stain-negative blood culture bottles according to the instructions of the manufacturer. Gram-stained and AO-stained slides were read by routine bench technicians. In addition, the bottles were subcultured to chocolate agar (incubated at 35°C in 5% CO₂ for 4 days), Sab-

ouraud agar (incubated at 28°C under aerobic conditions for 4 days), and blood agar (incubated at 35°C under anaerobic conditions for 4 days).

During an 18-month period (5 April 2001 to 4 October 2002), 18,424 sets of blood cultures were processed. Of these 2,074 (11.3%) were true positives. A total of 85 blood culture bottles were reported as positive by the system but were Gram stain negative. Upon subculturing, 73 of them showed no growth. Thus, the overall rate of false-positive blood culture bottles was 0.2% (0.3% for aerobic and 0.1% for anaerobic bottles). In previous studies, false-positive rates ranged from 0.11 to 1.8% with different BacT/ALERT blood culture media (3, 5–7). In our study, 12 (14.1%) of the 85 Gram stain-negative bottles grew microorganisms upon subculturing. AO staining detected microorganisms in 10 of these 12 cultures, namely, *Candida albicans* (3 isolates), *Candida tropicalis*, *Bacteroides ureolyticus*, coagulase-negative staphylococci, *Campylobacter fetus*, a *Veillonella* sp., and *Fusobacterium nucleatum* (two isolates in two blood cultures from the same patient). Only two positive cultures growing *Staphylococcus aureus* and *Proteus mirabilis* were missed by AO staining. While the *P. mirabilis* isolate was categorized as being of unknown clinical significance, the *Staphylococcus aureus* isolate was considered significant according to published criteria (8). *Staphylococcus aureus* was isolated from several blood cultures from the same patient, and thus, the infection would not have been missed without the subcultures. AO staining detected cocci in two blood culture bottles that remained negative upon subculturing. Since in both cases *Streptococcus pneumoniae* was isolated from the second bottle of the blood culture set, it is conceivable that AO staining detected nonviable *Streptococcus pneumoniae*. We conclude that AO staining can replace subcultures of Gram stain-negative blood cultures that are reported as positive. This is in agreement with the results of two previous studies which evaluated AO staining as a replacement for subculturing of false-positive blood cultures with the BACTEC NR 660. Besides eliminating the need to perform subcultures and thus saving time and money, AO staining proved particularly helpful in the early detection of candidemia. One-third of the microorganisms missed by Gram stain were yeasts, which are notoriously difficult to detect by Gram staining. In addition,

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AO staining can detect nonviable microorganisms and fastidious microorganisms like *Campylobacter* spp. and *Mycoplasma* spp., which are difficult or impossible to detect by Gram staining and do not grow when standard media and standard incubation conditions are used. Recently, notification of Gram stain results of blood cultures was reported to have a significantly higher impact on antimicrobial management of bloodstream infections than the release of antimicrobial susceptibility data (4). Accordingly, notification of AO stain results for Gram stain-negative blood cultures might have a significant impact on the clinical management of bloodstream infections, thus reducing costs from delayed or inappropriate antimicrobial therapy.

We thank Clarisse Straub for her assistance with the data management.

REFERENCES

1. Harrell, L. J., S. Mirrett, and L. B. Reller. 1994. Subcultures of BACTEC-positive but gram or acridine orange stain-negative NR 6A and 7A blood culture bottles are unnecessary. *Diagn. Microbiol. Infect. Dis.* **20**:121–125.
2. Hunter, J. S. 1993. Acridine orange staining as a replacement for subculturing of false-positive blood cultures with the BACTEC NR 660. *J. Clin. Microbiol.* **31**:465–466.
3. Mirrett, S., R. J. Everts, and L. B. Reller. 2001. Controlled comparison of original vented aerobic FAN medium with new nonvented BacT/ALERT FA medium for culturing blood. *J. Clin. Microbiol.* **39**:2098–2101.
4. Munson, E. L., D. J. Diekema, S. E. Beekmann, K. C. Chapin, and G. V. Doern. 2003. Detection and treatment of bloodstream infection: laboratory reporting and antimicrobial management. *J. Clin. Microbiol.* **41**:495–497.
5. Snyder, J. W., K. S. Benzing, G. K. Munier, G. D. Bostic, P. S. Bozigar, R. Hanna, and M. J. Zervos. 2000. Clinical comparison of nonvented aerobic BacT/Alert blood culture bottle and standard aerobic bottle for detection of microorganisms in blood. *J. Clin. Microbiol.* **38**:3864–3866.
6. Snyder, J. W., G. K. Munier, G. D. Bostic, P. S. Bozigar, and R. Hanna. 2002. Evaluation of a plastic nonvented aerobic blood culture bottle for use with the BacT/ALERT microbial detection system. *J. Clin. Microbiol.* **40**:4757–4759.
7. Weinstein, M. P., S. Mirrett, L. G. Reimer, M. L. Wilson, S. Smith-Elekes, C. R. Chuard, K. L. Joho, and L. B. Reller. 1995. Controlled evaluation of BacT/Alert standard aerobic and FAN aerobic blood culture bottles for detection of bacteremia and fungemia. *J. Clin. Microbiol.* **33**:978–981.
8. Weinstein, M. P., M. L. Towns, S. M. Quartey, S. Mirrett, L. G. Reimer, G. Parmigiani, and L. B. Reller. 1997. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. *Clin. Infect. Dis.* **24**:584–602.